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SEPARATION AND IDENTIFICATION OF ILLICIT HEROIN SAMPLES BY LIQUID CHROMATOGRAPHY USING AN ALUMINA AND C_{18} COUPLED COLUMN SYSTEM AND PHOTODIODE ARRAY DETECTION

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SUMMARY

The analysis of illicit heroin and opium samples on a coupled alumina and C_{18} column system is described. The compounds to be analysed can be divided into two groups: those with low pK_a values, such as caffeine, papaverine and noscapine, and those with high pK_a values, such as heroin, acetylcodeine, O^6-monoacetylmorphine, procaine, codeine, morphine and strychnine. The first group can best be separated on a C_{18} column, whereas alumina is more suitable for the second group. Previously reported criteria for choosing proper buffer systems for ion-exchange separations on alumina were used together with an iterative regressive optimization procedure developed in our laboratory. The system can be used with and without valve-switching, depending on the sample type.

The peak purity of the judicially important components heroin and O^6-monoacetylmorphine has been checked with a photodiode array detector and by use of advanced software.

INTRODUCTION

A number of analytical methods are in use for the identification of illicit heroin samples, from quite simple microchemical tests to mass spectrometry: combinations of different methods are often applied[1–3]. Because of the complex chemical composition of illicit heroin samples usually some kind of chromatography is used in the analysis. In addition to diacetylmorphine, such samples usually contain acetylcodeine, and frequently other alkaloids, such as noscapine, papaverine, (acetylthebaol), morphine and codeine, that originate from the starting materials, raw opium or non-purified morphine. Furthermore, the decomposition product O^6-monoacetylmorphine is invariably present, and in the South-East Asian "smoking heroin" the substances caffeine and strychnine are essential components. In addition, many trace

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components have been described[4,5]. The presence of a wide variety of non-narcotic drugs as cutting agents may also contribute to the wide use of chromatography.

Thin-layer chromatography is widely used for qualitative analysis, whereas gas chromatography (GC) has been the most common technique for quantitative determination. For the quantitation of heroin alone GC is suitable, although some problems caused by adsorption, heat instability, transesterification and solubility may arise[6,7]. For the simultaneous quantitative determination of the other compounds mentioned above, GC is not suitable unless derivatization is applied. This explains the increasing interest in the application of high-performance liquid chromatography (HPLC) for the analysis of illicit heroin samples. Many papers dealing with liquid chromatographic analysis of heroin have appeared. Extensive reviews have been presented by Gough and Baker[8] and Lurie and Wittwer[9]. In some cases silica was used as stationary phase, but most workers preferred C_{18}-bonded silica. A system using aminopropyl-bonded silica as stationary phase has also been described[10]. The description of numerous HPLC systems clearly demonstrates the requirement for a system capable of separating all the compounds that occur in illicit heroin samples in a single, preferably isocratic run.

Laurent *et al.* showed that alumina is a promising stationary phase in HPLC because of the many parameters that can be varied[11]. Alumina is an amphoteric ion-exchanger, which can be optimized for the separation of illicit heroin samples. The very basic compounds, present as cations under buffer conditions, can be separated in the ion-exchange mode. Basic solutes give problems on C_{18}-modified silica. For the separation of neutral compounds alumina is very suitable but on the other hand, they are easily separated on C_{18}. So, a combination of the two column types is an obvious choice. The types of illicit heroin encountered in the Netherlands, indicate that an optimum HPLC system should perform a baseline separation of heroin, O^6-monoacetylmorphine, acetylcodeine, papaverine, noscapine and acetylthebaol, as well as morphine and codeine, and preferably caffeine, procaine and strychnine. Separation should be achieved within 15 min. The practical development of such a system is described in this paper. Although the system has not been optimized for the analysis of opium samples, its usefulness for the quantitative determination of morphine in these samples is also demonstrated.

The chromatograms as obtained in such an in-depth analysis are complex, so there is no room for other compounds to be analysed simultaneously. Thus, a sample adulterated with some UV-absorbing components may have to be reanalysed on other columns or with other analytical techniques if total analysis is required. During this study, a photodiode array detector was used. Peak identification based on whole spectra is then possible, as well as peak deconvolution. In some cases, the use of this instrument allows quantitative determination of overlapping peaks.

HPLC has two significant disadvantages compared with GC. The first point concerns the lack of chromatographic plates when basic substances are chromatographed. Packed GC columns usually give separations with 5000–6000 plates and good peak shapes; the figures presented in the literature for HPLC separations on C_{18}-bonded silica usually show broad peaks and tailing[12–14]. Separations on silica or aminopropyl-bonded silica show better results[10,15]. The second point concerns the lack of selectivity in HPLC. In GC the chromatographic behaviour of almost all drugs has been described for the two stationary phases most frequently used in drug

analysis (SE-30 and OV-17), usually using Kovats indices[16,17]. No serious interference with heroin determination from other substances is expected[18]. For HPLC few data are available. Lurie[19] mentioned the restricted selectivity of HPLC systems, and proposed that detection be carried out at two different wavelengths, which significantly increases the separating power[20].

Reliable quantitative data can be obtained when there is no doubt about the purity of the eluting compound. Especially in countries where the penalties for drug offences are directly connected to quantitative data, such a purity check (or the use of a second quantitative method) must be considered. In this paper the use of the diode array detector for this confirmation is described.

EXPERIMENTAL

The liquid chromatograph included an M6000A pump, an M440 UV detector, both from Millipore Waters Chromatography Division (Milford, MA, U.S.A.) and a Rheodyne 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, U.S.A.). A Perkin-Elmer Model 3000 fluorescence detector (Perkin-Elmer, Norwalk, CT, U.S.A.) was coupled in series with a UV detector to monitor the column effluent (excitation wavelength, 260 nm; emission wavelength 400 nm; slits, 10 nm).

The alumina column (25 cm × 4.6 mm I.D., Valco type) was packed in the laboratory with Spherisorb A5Y (Phase Separations, Queensferry, Clwyd, U.K.). The alumina was used without any pretreatment. The column was packed with methanol as slurry and displacement liquid, and then washed with water and equilibrated with the starting buffer.

The C_{18}-modified silica was obtained from Shandon Southern Products (Runcorn, Cheshire, U.K.). The column dimensions (45 × 2.1 mm I.D.) were derived from the optimization of the separation (see Results and Discussion). The C_{18} column was packed in the laboratory with methanol as slurry and displacement liquid.

A six-port Valco valve was used for column-switching between the alumina and C_{18} columns (the instrumental set-up is described in Results and Discussion).

The organic solvents methanol and acetonitrile were obtained from Rathburn Chemicals (Walkerburn, U.K.). Distilled water was further purified by a MilliQ system (Millipore, Molsheim, France).

All drugs and samples of illicit heroin and opium were obtained from the Forensic Science Laboratory of the Ministery of Justice, Rijswijk, The Netherlands, and are listed in Table I.

Samples for chromatography were prepared by dissolving 10 mg in 5 ml of eluent in an ultrasonic bath. Filtration prior to injection was necessary.

A Hewlett-Packard 1040A fast-scanning linear diode array detector was used, connected to an HP-85 desktop computer equipped with input/output, plotter, printer, mass storage and advanced programming ROMs, 16 Kbyte additional memory and an HP-IB IEEE-488 interface (Hewlett-Packard, Waldbronn, F.R.G.). The HP-7470A graphics plotter and the HP-82910 M dual 5¼ in. flexible disk-drive were connected to the HP-85 via the HP-IB bus interface.

The software for handling the 1040A detector, the storing of data and plotting was the standard software version I (Evalu I) supplied by Hewlett-Packard. Additional software was developed in HP-Basic on the HP-85 to present the maximal

TABLE I

RETENTION TIMES OF REFERENCE COMPOUNDS

Chromatographic conditions: 28% methanol, 17% acetonitrile, 55% citrate/TMS (0.01 *M*) buffer; pH 6; 1 ml/min.

Reference compound	*Retention time*
Meconic acid	2.05
Dipyrone	2.30
Caffeine	2.96
Meconine	3.15
Dipyrone (sec. peak)	3.20
Papaverine	3.61
Noscapine	5.96
Acetylthebaol	7.44
Alpha-thebaol	7.57
Heroin	8.72
Acetylcodeine	10.01
O^3-Monoacetylmorphine	10.39
Cryptopine	10.83
O^6-Monoacetylmorphine	11.00
Procaine	11.24
Thebaine	12.16
Codeine	12.23
Strychnine	13.31
Morphine	14.35
3,6-Dimethoxyphenanthrene-4,5-epoxide	15.73

absorbance chromatogram, normalized spectra-plot and selected multi-wavelength chromatogram as described in the literature[21]. The program "KU-curve Resolution (KU-CR-3) written in HP-Basic for an HP-85, and to be run with the HP1040A diode array detector standard software" was obtained from B.G.M. Vandeginste (Catholic University, Nijmegen, The Netherlands)[22]. The optimization software, developed by A. Drouen[23] was run on a PC 350 Digital Equipment computer (Maynard, MA, U.S.A.) connected to an HP7470A graphics plotter.

RESULTS AND DISCUSSION

Design and optimization of the column system

The selected compounds to be analysed can be divided into two groups: those with low pK_a values, such as caffeine, papaverine and noscapine, and those with high pK_a values, such as heroin, acetylcodeine, O^6-monoacetylmorphine, procaine, codeine, morphine and strychnine. Laurent *et al.*[11] showed that separation of solutes with high pK_a values can be obtained on alumina; the retention and the selectivity can be influenced by the addition of organic modifier to aqueous buffer systems using alumina as cation-exchanger. However, the addition of organic modifier moves the retention of the solutes with low pK_a values to the column void-volume, so selectivity then drops very quickly. An elegant solution to this problem is to use a combined

column system containing alumina and C_{18} material. The idea is to design a mobile phase system where the solutes with low pK_a values are temporarily kept on the C_{18} column, by applying a switching valve (see Fig. 1). After the elution of the very basic solutes from the alumina column the neutral materials are separated and eluted from the C_{18} column.

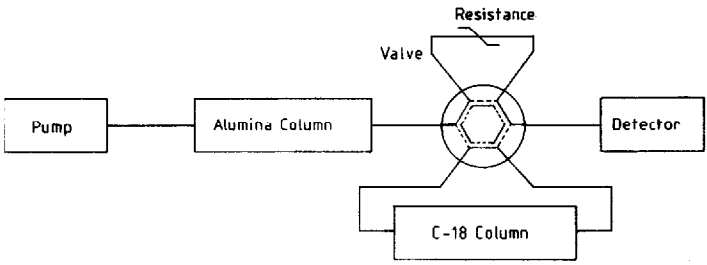

Fig. 1. Column-coupling configuration as used for the separation of heroin and opium samples.

The other option is to use both columns in series, the solutes with low pK_a values being directly separated on the C_{18} column, while the solutes with high pK_a values are simultaneously separated on the alumina column. These basic solutes are more or less unretained on the C_{18} column; alternatively, they can be lead directly to the detector via the switching valve.

Optimization on the alumina column

Criteria for choosing appropriate buffer systems for ion-exchange separations on alumina have been described by Laurent *et al.*[24]. Tetramethylammonium hydroxide (TMA) in a concentration of 0.01 *M* was chosen as counter-ion because of its good solubility in methanol and acetonitrile. Other homologues can be considered if the retention is to be manipulated. Usually, retention increases as the alkyl chain length of the buffer ion increases. The pH of the buffer was fixed at 6.0 with citric acid. This value was chosen from previous experience of the behaviour of the pK_a values of the solutes and the zero point of charge (ZPC) of alumina. The concept of ZPC in relation to the buffer anion and pH is described elsewhere[25].

The iterative regressive optimization procedure described by Drouen *et al.*[23] was used to optimize the type and concentration of the organic modifier for the separation of the high pK_a solutes on the alumina column. Essentially, this procedure first formulates a linear relation between the retention and optimization parameters on the basis of a few initial runs. Six different mobile phases containing methanol (45%, 60% and 75%) or acetonitrile (30%, 45% and 60%) in the aqueous TMA–citric acid buffer were used as initial compositions in order to collect chromatographic input data for the calculation of the phase-selection diagrams. The most promising system out of the nine possible combinations was kept for further investigation (see Fig. 2A). The binary starting conditions are methanol–buffer (45:55) and acetonitrile–buffer (45:55). The optimization procedure starts by assuming linear be-

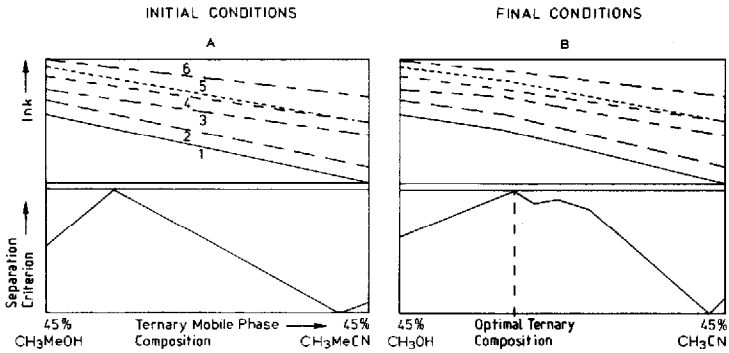

Fig. 2. Solvent-selection diagram for the optimization of six solutes on the alumina column: heroin (1), acetylcodeine (2), procaine (3), codeine (4), morphine (5), strychnine (6).

haviour over the ternary mixture methanol–acetonitrile–buffer. In Fig. 2A (lower part) the separation criterion is presented. This criterion is used to determine the quality of the chromatogram and is the minimum value of the resolution of the least separated peak pair ($R_{s,min}$). The optimum mobile phase composition can be found at the highest point. Behaviour that is not truly linear can be corrected in an iterative way by taking more data points. The end result is presented in Fig. 2B. The optimum mobile phase composition is 17% acetonitrile, 28% methanol and 55% buffer. The resulting chromatogram is shown in Fig. 3A.

The retention behaviour of the low pK_a solutes was investigated on the C_{18} column using the methanol–water and acetonitrile–water mixtures derived for the alumina column. The measurements resulted in an estimate of the column length needed firstly to store the solutes on the column (in the column-switching system of Fig. 1) and secondly to separate the solutes afterwards. During the development of the system, it turned out that the length of the C_{18} column could be designed such that no switching valve was necessary. The low pK_a solutes first pass the alumina column unretained and are separated on the C_{18} column, while at the same time the high pK_a solutes are separated on the alumina column, and pass the C_{18} column more or less unretained.

Application of the system

The retention times of several heroin- and/or opium-related substances obtained for the non-switched system are listed in Table I. A chromatogram obtained with the columns in the switched mode is shown in Fig. 3A, and a chromatogram with the columns in series is shown in Fig. 3B. From the compounds with the high pK_a values a low retention on C_{18} is observed for heroin, acetylcodeine, codeine and strychnine, whereas procaine and morphine do not show additional retention. Fig. 4 shows the chromatogram of the illicit heroin sample no 1. This sample, originating from South-West Asia, was relatively pure. No adulterants were detected, and the following components could be identified: papaverine, noscapine, acetylthebaol, heroin, acetylcodeine and O^6-monoacetylmorphine. Small peaks that eluted before and just after the papaverine peak could not be identified owing to the lack of standards, although their spectra are available.

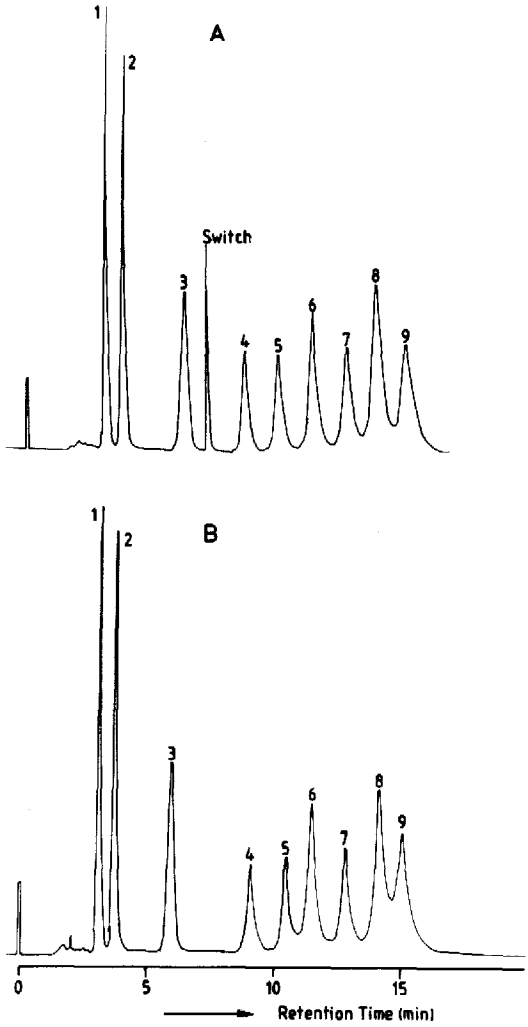

Fig. 3. Chromatograms of reference compounds; Conditions: 28% methanol, 17% acetonitrile and 55% citrate/TMA (0.01 *M*) buffer; pH 6; flow-rate 1.0 ml/min; UV-detection at 254 nm. Solutes: caffeine (1), papaverine (2), noscapine (3), heroin (4), acetylcodeine (5), procaine (6), codeine (7), strychnine (8) and morphine (9). (A) Alumina and C_{18} column with switching; (B) alumina and C_{18} column in series.

Column-switching is not necessary because there are no non-polar substances present with long retention times on the C_{18} column. Peak purity was confirmed by the curve resolution program KU-CR-3 for the judicially important components heroin and O^6-monoacetylmorphine. A fast recording of complete spectra at short time intervals during the elution of the peaks yielded a two-dimensional data-matrix. These spectra are linear combinations of the spectra of pure solutes if peak overlapping occurs. Because of the linear additivity of the signals, the number of coeluting compounds can be determined by a principal component analysis (PCA). Also, the

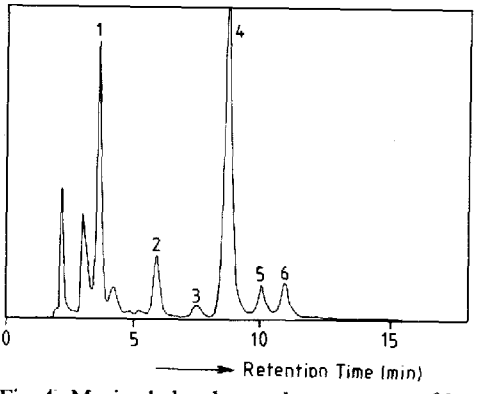

Fig. 4. Maximal absorbance chromatogram of heroin sample No. 1. Solutes: papaverine (1), noscapine (2), acetylthebaol (3), heroin (4), acetylcodeine (5), O^6-monoacetylmorphine (6).

pure spectra can be derived from such a data-matrix if the peak cluster contains a maximum of three components. Resolved elution profiles can then be reconstructed using estimated "pure" spectra.

To process the data, a PCA is first performed on a peak (cluster) to estimate the total number of solutes in the cluster. The result of the PCA is a table containing a number of eigenvalues. The decision on how many eigenvalues are relevant to the system can be best taken using the residual standard deviation when the experimental error is known. From this analysis, it was clear that only one compound was present. This was confirmed by the reconstructed elution profile and the estimated pure spectrum (for heroin, see Fig. 5). The system has been applied to another heroin sample (No. 2), also originating from South-West Asia. In contrast to the previous sample, this one was "cut" with the analgesic dipyrone. Fig. 6A shows the chromatogram on the alumina column. Clearly, this column alone is unable to give a good separation of all compounds present. Fig. 6B illustrates that better separation in the first part of the chromatogram is obtained on a switched system. When both columns are used in series, acetylthebaol is also eluted, as a single peak (Fig. 6C). This peak is also clearly seen on the chromatogram obtained with the fluorimeter as detector (Fig. 6D). In addition to acetylthebaol, several minor components that are not retained by alumina, are also detected. These may contribute to a so-called "fingerprint" of the sample, which can be used for comparison purposes.

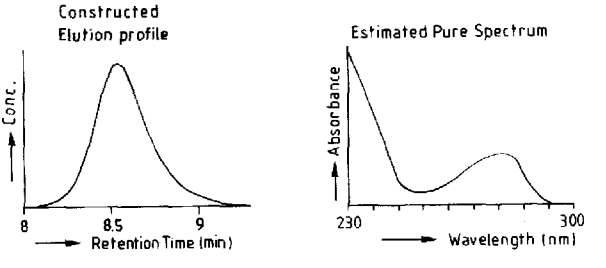

Fig. 5. Constructed elution profile and estimated pure spectrum of the heroin peak in sample No. 1 (peak 4).

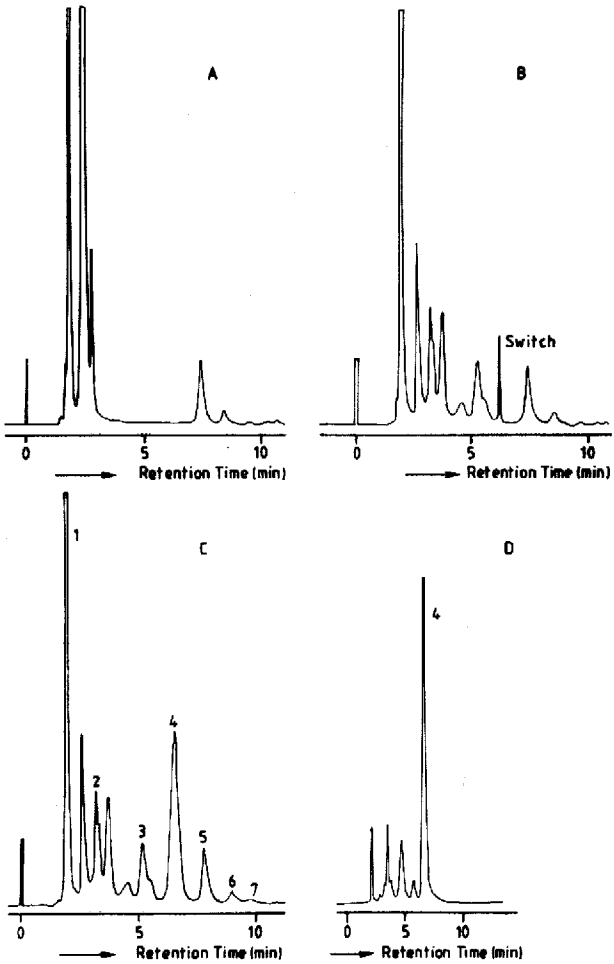

Fig. 6. Chromatograms of heroin sample No. 2. Chromatographic conditions as in Fig. 3. Solutes: dipyrone (1), papaverine (2), noscapine (3), acetylthebaol (4), heroin (5), acetylcodeine (6), O^6-monoacetylmorphine (7). (A) Alumina column; (B) alumina–C_{18} switched column system; (C) alumina–C_{18} columns in series; (D) alumina–C_{18} columns in series with fluorimetric detection.

Application of the system to an opium sample

Opium is a natural product with a more complex matrix than heroin samples. For opium analysis it is advantageous to use the column-switching system to avoid interference by late-eluting compounds on the C_{18} column with solutes leaving the alumina column. The chromatogram is shown in Fig. 7. The switching point is indicated in the figure. The compounds identified were meconic acid, papaverine, noscapine, cryptopine, thebaine, codeine and morphine. The method is well suited for the analysis of morphine in opium samples. Morphine elutes within 15 min. Fluorimetric detection yields several peaks; the strongest of them could be identified as meconine.

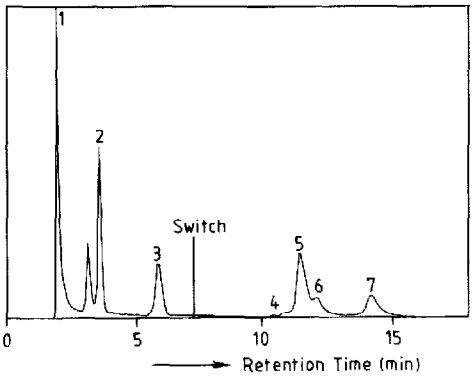

Fig. 7. Maximal absorbance chromatogram of an opium sample. Solutes: meconic acid (1), papaverine (2), noscapine (3), cryptopine (4), thebaine (5), codeine (6) and morphine (7).

CONCLUSIONS

The method described in this paper allows an easy and straightforward analysis of illicit heroin and opium samples. The system proved to be stable over a long period. The sample preparation is easy and suitable for both heroin and opium samples, owing to the good solubility in the eluent.

Combining HPLC with a photodiode array detector and use of the advanced software to check the purity of the eluted peaks makes the method adequate for forensic purposes. Although the mobile phase was optimized for the columns coupled in series, column-switching can be used successfully for "cut" heroin and for opium samples. The important constituents of heroin or opium samples can be reliably quantitated.

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